# Virulence of Entomopathogenic Nematodes to Pecan Weevil Larvae, *Curculio caryae* (Coleoptera: Curculionidae), in the Laboratory

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**ABSTRACT** The pecan weevil, *Curculio caryae* (Horn), is a key pest of pecans in the Southeast. Entomopathogenic nematodes have been shown to be pathogenic toward the larval stage of this pest. Before this research, only three species of nematodes had been tested against pecan weevil larvae. In this study, the virulence of the following nine species and 15 strains of nematodes toward fourth-instar pecan weevil was tested: *Heterorhabditis bacteriophora* Poinar (Baine, HP88, Oswego, NJ1, and Tf strains), *H. indica* Poinar, Karunakar & David (original and Hom1 strains), *H. marelatus* Liu & Berry (IN and Point Reyes strains), *H. megidis* Poinar, Jackson & Klein (UK211 strain), *H. zealandica* Poinar (NZH3 strain), *Steinernema riobrave* Cabanillas, Poinar & Raulston (355 strain), *S. carpocapsae* (Weiser) (All strain), *S. feltiae* (Filipjev) (SN strain), and *S. glaseri* (Steiner) (NJ43 strain). No significant difference in virulence was detected among nematode species or strains. Nematode-induced mortality was not significantly greater than control mortality (in any of the experiments conducted) for the following nematodes: *H.* bacteriophora (Baine), *H. zealandica* (NZH3), *S. carpocapsae* (All), *S. feltiae* (SN), *S. glaseri* (NJ43), and *S. riobrave* (355). All other nematodes caused greater mortality than the control in at least one experiment. *Heterorhabditis megidis* (UK211) but not *H. indica* (original) displayed a positive linear relationship between nematode concentration and larval mortality. Results suggested that, as pecan weevil larvae age, they may have become more resistant to infection with entomopathogenic nematodes.

**KEY WORDS** *Steinernema*, *Heterorhabditis*, biological control, *Curculio caryae*, entomopathogenic nematode, virulence

THE PECAN WEEVIL, *Curculio caryae* (Horn), is a major pest of pecans throughout the Southeast (Mizell 1985). The insects have a 2- or 3-yr life cycle (Harris 1985). Adults emerge from soil in late July–August, feed on developing nuts, and oviposit into the nuts after dough stage (Harris 1985). Larval development is completed within the nut, and fourth instars drop to the soil where they burrow to a depth of 8–25 cm, form a puparium, and overwinter. The following year ≈90% of the larvae pupate and spend the next 9 mo in the soil as adults (Harris 1985). The remaining 10% of the population spend 2 yr in the soil as larvae and emerge as adults in the third year (Harris 1985).

Current control recommendations for the pecan weevil consist solely of above-ground applications of carbaryl to suppress adults (Harris 1999, Ellis et al. 2000). Because of the problems associated with aphid resurgence (Dutcher and Payne 1985), as well as other environmental and regulatory concerns, research on developing alternative control strategies is warranted. Entomopathogenic nematodes are one of the potential alternatives.

Entomopathogenic nematodes genera *Steinernema* and *Heterorhabditis* are obligate parasites of insects (Kaya and Gaugler 1993). These nematodes are mutualistically associated with bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp. for steinernematids and heterorhabditids, respectively). Infective juveniles, the only free-living stage, enter hosts through natural openings (mouth, anus, and spiracles), or in case of heterorhabditids, also through the cuticle (Poinar 1990). After entering the host's hemocoel, the nematodes release their symbiotic bacteria, which are primarily responsible for killing the host and providing the nematodes with nutrition and defense against secondary invaders (Poinar 1990). The nematodes molt and complete two to three generations within the host after which infective juveniles are released to search out new hosts (Kaya and Gaugler 1993).

Entomopathogenic nematodes are effective biocontrol agents of a variety of economically important insect pests (Klein 1990). Because of sensitivity to UV light and desiccation, entomopathogenic nematodes have been most efficacious in soil or other protected environments (Kaya and Gaugler 1993). Biological control with entomopathogenic nematodes has been particularly successful against certain weevil species that spend a large portion of their life cycle in the soil, e.g., the Diaprepes root weevil *Diaprepes abbreviatus* (L.) (Duncan and McCoy 1996, Duncan et al. 1996, Bullock et al. 1999) and the black vine weevil, *Otio-*

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**Table 1. Entomopathogenic nematodes used in this study**

| Species | Strain | Source | Abbreviation |
|---|---|---|---|
| *Heterorhabditis bacteriophora* Poinar | Baine | K. Nguyen, University of Florida, Gainesville | HbBai |
| *H. bacteriophora* | NJ1 | Bio Integrated Technology S. R. L. Perugia, Italy | HbNj1 |
| *H. bacteriophora* | HP88 | MicroBio, L. T. D., Cambridge, U. K. | HbHp8 |
| *H. bacteriophora* | Oswego | E. Shields and M. Villani, Cornell University, Ithaca | HbOsw |
| *H. bacteriophora* | TF | R. Gaugler, Rutgers University, New Brunswick | HbTf |
| *H. indica* Poinar, Karanukar & David | Original isolate | K. Nguyen, University of Florida, Gainesville | HiOrg |
| *H. indica* | Hom1 | Integrated BioControl Systems, Lawrenceburg, IN | HiHom |
| *H. marelautus* Liu & Berry | IN | Integrated BioControl Systems, Lawrenceburg, IN | HmIn |
| *H. marelatus* | Point Reyes | P. Stock, University of California, Davis | HmPoi |
| *H. megidis* Poinar, Jackson & Klein | UK211 | MicroBio, L. T. D., Cambridge, U. K. | HmUk2 |
| *H. zealandica* Poinar | NZH3 | P. Stock, University of California, Davis | HzNzh |
| *Steinernema riobrave* Cabanillas, Poinar & Raulston | 355 | Thermo Trilogy Columbia, MD | Sr355 |
| *S. carpocapsae* (Weiser) | All | K. Nguyen, University of Florida, Gainesville | ScAll |
| *S. feltiae* (Filipjev) | SN | K. Nguyen, University of Florida, Gainesville | SfSn |
| *S. glaseri* (Steiner) | NJ43 | K. Nguyen, University of Florida, Gainesville | SgNj4 |

*rhynchus sulcatus* (F.) (Bedding and Miller 1981, Simons 1981, Shanks and Agudelo-Silva 1990).

Based on previous success suppressing other weevils, and the time that pecan weevil spends in the soil, we may expect the pecan weevil to be a good candidate for control with entomopathogenic nematodes. Entomopathogenic nematodes have been reported to occur naturally in pecan weevil larvae (Harp and Van Cleave 1976, Nyczepir et al. 1992). However, levels of pecan weevil control with nematodes in laboratory and field studies have been low (Nyczepir et al. 1992, Smith et al. 1993) except when extremely high rates are used (Tedders 1973).

Efficacy of entomopathogenic nematodes is dependent on matching the most effective nematode with the target pest (Georgis and Gaugler 1991). Certain entomopathogenic nematode species may be highly effective against a particular pest, whereas others may be only moderately effective or ineffective (Georgis and Gaugler 1991, Mannion and Jansson 1992, Patterson Stark and Lacey 1999). Hominick et al. (1997) recognize 30 species of entomopathogenic nematodes and there are numerous strains of each (Poinar 1990). Before this research, only three species had been tested for pathogenicity toward the pecan weevil: *H. bacteriophora* Poinar, *S. carpocapsae* (Weiser), and *S. feltiae* (Filipjev) (Tedders 1973, Nyczepir et al. 1992, Smith et al. 1993). Laboratory testing of additional species and strains may lead to the identification of nematodes with superior virulence toward the pecan weevil. The primary objective of this study was to compare the virulence of nine entomopathogenic nematode species and 15 strains under laboratory conditions. Additionally, dose–response relationships between two nematode species and pecan weevil mortality were investigated. After experimentation, it appeared that higher mortality occurred in younger larvae (used in earlier experiments) relative to larvae that had been stored longer. Therefore, a third objective was included to confirm the change in weevil mortality over time, and to begin to elucidate its cause.

## Materials and Methods

**Nematodes, Insects, and Experimental Parameters.** The sources of nematode cultures are listed in Table 1. All nematodes for all experiments were reared in parallel on last instar *Galleria mellonella* (L.) at 25°C, according to procedures described in Woodring and Kaya (1988). *Galleria mellonella* larvae were obtained from Sunfish Bait (Webster, WI). After harvesting, nematodes were stored at 13°C for <3 wk before experimentation. Pecan weevil larvae (fourth instar) were collected from infested nuts and stored in sterile (autoclaved) soil at 4–10°C. Mortality was monitored in 588 of the larvae during the first 59–70 d of storage (four replicates). Experiments were conducted in plastic cups (Bioserv, Frenchtown, NJ) based on procedures described by Shapiro et al. (1999). Cups (3–4 cm i.d., 3.5 cm deep) were filled with (oven-dried) soil from the USDA-ARS pecan orchard (Byron, GA) and contained one larva each. The soil was a loamy sand with the percentage sand:silt:clay = 84:10:6, pH = 6.1, and organic matter = 2.8% by weight. Nematodes were pipetted onto the soil surface of each cup in 0.5 ml of water so that the final moisture was standardized at field capacity (14% moisture). All experiments contained an untreated control (only water added) and were arranged in completely randomized designs.

**Comparing Virulence Among Nematodes.** Although all the experiments described below provided some information on relative virulence among nematodes, the following two experiments were designed to address this objective directly.

*Experiment 1.* Virulence toward pecan weevil larvae was tested in 13 strains (comprising nine species) of entomopathogenic nematodes: *H. bacteriophora* (Baine, NJ1, and HP88 strains), *H. indica* Poinar, Karunakar & David (original and Hom1 strains), *H. marelatus* Liu & Berry (IN and Point Reyes strains), *H. megidis* Poinar, Jackson & Klein, *H. zealandica* Poinar, *Steinernema riobrave* Cabanillas, Poinar & Raulston, *S. carpocapsae, S. feltiae,* and *S. glaseri* (Steiner).

There were four replicates of 10 cups per treatment (strain). Approximately 500 infective juveniles were

applied to each cup, and larval mortality was recorded after 13 d of incubation at 25°C. This experiment was repeated (i.e., two trials); the second trial was initiated eight days after the first one began. Pecan weevil larvae were stored 11–30 d before experimentation.

*Experiment 2.* The five nematode species that caused the highest pecan weevil mortality in the first experiment were compared further. The strain causing the highest mortality within each of these species was included: *H. bacteriophora* (NJ1), *H. indica* (original), *H. marelatus* (Point Reyes), *H. megidis*, and *S. riobrave*. Two additional (untested) strains were also included in the experiment: *H. bacteriophora* Oswego and TF strains. The Oswego strain was added due to its efficacy in controlling the alfalfa snout beetle, *Otiorhynchus ligustici* (L.) (Shields et al. 1999), and the TF strain was added due to its exceptional reproductive capacity (Randy Gaugler, Rutgers University, personal communication). There were four replicates of 10 cups per treatment. Approximately 500 infective juveniles were applied to each cup and larval mortality was recorded after 14 d of incubation at 25°C. This experiment was repeated except that the second trial contained three replicates of 10 cups per treatment. The second trial was initiated three days after the first one began. Pecan weevil larvae used in this experiment were older than in the first experiment (storage time was 74–89 d before experimentation). Nematodes in this experiment had undergone one additional passage in *G. mellonella* since the first experiment.

**Nematode Concentration Effects (Experiment 3).** The relationship between nematode concentration and pecan weevil mortality was tested in *H. indica* (original) and *H. megidis*.

These two nematodes were chosen because they were the only ones that caused greater larval mortality than the control in experiment 2. Experimental parameters were the same as described previously except that a range of concentrations was applied: 0, 100, 250, 500, 1,000, and 1,500 infective juveniles per cup. There were three replicates of 10 cups for each concentration and each nematode. Larval mortality was recorded after 13 d of incubation at 25°C. Pecan weevil larvae were stored 124–135 d before experimentation. Nematodes in this experiment had undergone one additional passage in *G. mellonella* since the first experiment.

**Changes in Larval Mortality Over Time.** After experimentation it was noticed that nematode-induced mortality was greater in the first experiment than in the second. This was first verified by comparing larval mortality caused by the five nematode treatments that were common to both experiments. An additional experiment was then devised to address this issue.

*Experiment 4.* This experiment tested whether or not nematodes may have lost virulence over time. I hypothesized that if the nematodes had lost virulence then their ability to kill other pests would also be reduced. Therefore, an additional host—the Diaprepes root weevil —was included to see if mortality would be comparable to previous experiments conducted under similar conditions (Shapiro et al. 1999, Shapiro and McCoy 2000). Treatments included *H. indica* (original) tested against pecan weevil, *H. indica* (original) tested against 10th- and 11th-instar Diaprepes root weevil, *H. megidis* tested against pecan weevil, and two untreated controls—one for each insect. There were three replicates of 10 cups for each treatment. Larval mortality was recorded after 13 d of incubation at 25°C. Pecan weevil larvae were stored 124–135 d before experimentation. Nematodes in this experiment had undergone one additional passage since the first experiment.

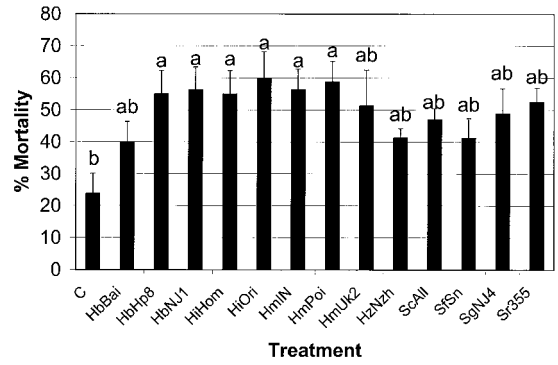


**Fig. 1.** Pecan weevil mortality after exposure to 13 entomopathogenic nematode strains. Different letters above bars indicate statistical significance ($P < 0.05$). See text for description of nematode treatment abbreviations; C, control (water).

**Statistical Analyses.** Analysis of variance (ANOVA) and Tukey's multiple range test (SAS Institute 1985) was used to test for differences among treatments in all experiments. All data recorded as percentages were transformed (arcsine of the square root) before analysis. Within each experiment, when no significant interaction was detected between trial and treatment ($P > 0.05$), data were pooled among trials. The relationship between nematode concentration and pecan weevil mortality in experiment 3 was analyzed with linear regression (SAS Institute 1985). *T*-tests (SAS Institute 1985) were used to determine if average pecan weevil mortality differed between experiment 1 and experiment 2; the nematode treatments that were common to both experiments were compared as were the controls in each experiment.

## Results

**Comparing Virulence Among Nematodes.** Treatment differences were detected through ANOVA in experiments 1 and 2 ($F = 2.54$; df = 13, 81; $P = 0.0055$, and $F = 3.2$; df = 7, 37; $P = 0.0095$, for experiments 1 and 2, respectively). In experiment 1, the following nematodes caused greater mortality compared with the control: *H. bacteriophora* (NJ1 and HP88), *H. indica* (original and Hom1), and *H. marelatus* (IN and Point Reyes) (Fig. 1). No significant differences in virulence were detected among the nematode strains and species (Fig. 1). In experiment 2 only *H. indica* (original) and *H. megidis* caused greater mortality

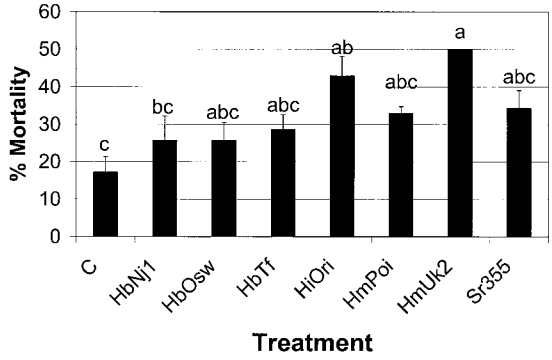


**Fig. 2.** Pecan weevil mortality after exposure to seven entomopathogenic nematode strains. Different letters above bars indicate statistical significance ($P < 0.05$). See text for description of nematode treatment abbreviations; C, control (water).

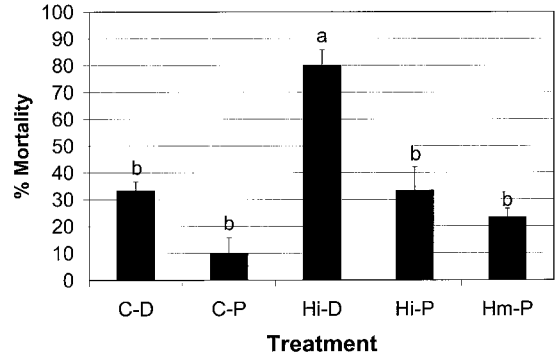


**Fig. 4.** Larval mortality after exposure to entomopathogenic nematodes or a water control. Different letters above bars indicate statistical significance ($P < 0.05$). C, control; D, Diaprepes root weevil; Hi, *Heterorhabditis indica* (original); Hm, *H. megidis* (UK211), P, pecan weevil.

than in the control (Fig. 2). No significant differences in virulence were detected among nematode strains or species (Fig. 2).

**Concentration Effects.** Pecan weevil mortality from *H. megidis* and *H. indica* (original) at different nematode concentrations is depicted in Fig. 3. *Heterorhabditis megidis* displayed a positive linear relationship between nematode concentration and larval mortality ($P = 0.0090$, $y = 0.001x + 1.6$, $R^2 = 0.355$). No significant linear response to nematode concentration was detected for *H. indica* ($P = 0.7925$). An interaction between nematode concentration and species was detected ($F = 3.79$; df = 4, 20; $P = 0.0188$). Only the nematode concentration of 1,500 *H. megidis* caused mortality significantly greater than in the control ($F = 2.46$; df = 10, 22; $P = 0.0374$, and Tukey's test).

**Changes in Larval Mortality Over Time.** The five nematode treatments that were common to both experiment 1 and 2 caused higher pecan weevil mortality in experiment 1 ($T = 4.39$, df = 65, $P = 0.0001$); mean percentage mortalities (±SE) were 55.3 ± 3.4 and 35.5 ± 2.5, for experiment 1 and 2, respectively. There was no significant interaction between treatments and experiments ($P = 0.3438$). Nematode-induced mortality was also greater in experiment 1 than experiment 2 when Abbott's formula (Abbott 1925) was applied to correct for mortality in the control ($T = 3.3$, df = 8, $P = 0.01$); mean percentage mortalities were 42.0 ± 2.2 and 24.0 ± 5.0, for experiment 1 and 2, respectively. Control mortality did not differ significantly between experiment 1 and 2 ($T = 0.630$, df = 13, $P = 0.5396$); mean percentage mortalities were 23.8 ± 6.3 and 17.1 ± 4.2, for experiment 1 and 2, respectively. Pecan weevil mortality also declined when virulence of *H. indica* and *H. megidis* was compared in experiment 1 versus experiment 3 and 4 ($T = 0.2.8$, df = 16, $P = 0.0125$). Survival of weevil larvae averaged 91.67 ± 4.5% during the first 59–70 d of storage.

Treatment differences were detected in experiment 4 ($F = 11.48$; df = 5, 12; $P = 0.0003$). *Heterorhabditis indica*-induced mortality of Diaprepes root weevils was greater than *H. indica*-induced mortality of pecan weevils and all other treatments (which were not different from each other) (Fig. 4).

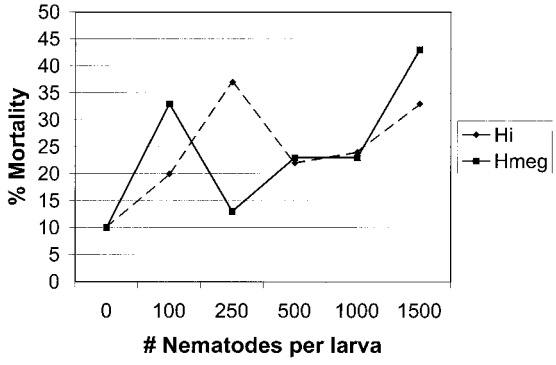


**Fig. 3.** Effect of nematode concentration on pecan weevil larval mortality. Hi, *Heterorhabditis indica* (original); Hmeg, *H. megidis* (UK211).

## Discussion

Nematode virulence toward the pecan weevil did not differ greatly among species. Smith et al. (1993) also did not detect virulence differences toward the pecan weevil when comparing *H. bacteriophora*, *S. feltiae*, and *S. carpocapsae*. Various studies that used many of the same nematode species as this study, and had assay designs similar to the one presented here, have shown substantial differences in virulence toward other insects, e.g., the Diaprepes root weevil (Shapiro et al. 1999, Shapiro and McCoy 2000), the western cherry fruit fly, *Rhagoletis indifferens* Curran (Patterson Stark and Lacey 1999), and various Lepidoptera (Morris and Converse 1991).

The level of nematode-induced pecan weevil mortality reported among studies varies. Nyczepir et al. (1992) and Smith et al. (1993) detected low mortality (<25% when Abbott's formula was applied) in studies conducted under controlled conditions. The mortality

observed in the study presented here was considerably higher. For example, contrary to our results, Smith et al. (1993) did not detect any control in *H. bacteriophora* (HP88). Similar to our study, however, Smith et al. (1993) reported no significant difference between mortality caused by *S. carpocapsae* (All) and the control. Various experimental conditions (Kaya 1990, Georgis and Gaugler 1991) must account for some of the variation in results. Indeed, Nyczepir et al. (1992) cite low soil moisture as one potential cause for the low mortality they observed. Tedders (1973) observed 67% mortality in a field experiment, but the rate of application was exceedingly high (>8,000 per $cm^2$) and thus not comparable to other studies.

The data showed clearly that *H. indica* is more virulent toward the Diaprepes root weevil than to the pecan weevil. Relative to our observations in the pecan weevil, other laboratory studies have reported substantially higher nematode-induced mortality in other insects, e.g., the Diaprepes root weevil (Shapiro and McCoy 2000), the black vine weevil (Schirocki and Hague 1997), and the Japanese beetle (Mannion et al. 2000). Although differences in experimental parameters limit comparisons among these studies, the bulk of the literature support the premise that pecan weevil larvae are not as susceptible as various other weevil and other Coleoptera larvae (see Klein 1990).

In dose–response experiments, a positive relationship between nematode concentration and larval mortality was observed in only one of the two nematodes tested, and even in that case the dose effect did not account for a large proportion of the variation in mortality. For some pathogens it is possible that once a mortality-producing threshold dose is reached, increased pathogen units will not produce further mortality (Tanada and Fuxa 1987). Smith et al. (1993) also did not detect a dose–response relationship when testing *H. bacteriophora*, *S. carpocapsae*, and *S. feltiae* against the pecan weevil.

Pecan weevil mortality decreased after the first experiment. Possible explanations include loss of nematode virulence over time (attenuation), a higher prevalence of nonintroduced diseases in younger larvae (used in the first experiment), or development of resistance to nematodes in older larvae. Serial passage can cause reduction in beneficial traits in various pathogens (Tanada and Fuxa 1987, Tanada and Kaya 1993) including entomopathogenic nematodes (Shapiro et al. 1996a, Stuart and Gaugler 1996). For example, in *H. bacteriophora*, reduction of beneficial traits (heat tolerance) has been observed after eight passages through *G. mellonella* (Shapiro et al. 1996a). However, the reduced pecan weevil mortality observed in our later experiments was not due to attenuation. The likelihood of attenuation was low because there was only a difference of one passage between nematodes in experiment 1 and 2. Furthermore, my data did not support the hypothesis that nematodes attenuated; nematode-induced mortality in Diaprepes root weevils was comparable to levels reported in similar studies (Shapiro et al.1999, Shapiro and McCoy 2000).

The observed decline in pecan weevil mortality over time was probably not due to unequal prevalence of nonintroduced diseases among weevils in the experiments. If the first experiment had a disproportionately high level of weevils containing lethal doses of nonintroduced pathogens, then one would have expected to observe significant differences in control mortality between experiment 1 and 2, but this was not the case. Alternatively, we would expect a large proportion of weevils to have died in storage, but this was also not the case.

The reduced nematode virulence observed in later experiments may have been due to development of resistance in older pecan weevil larvae. Entomopathogenic nematode virulence can depend on the age and stage of the insect. For example, in some hosts, susceptibility to entomopathogenic nematodes decreases as larval stage increases (Glazer 1992, Shapiro et al. 1999). Additionally, susceptibility can change according to the age of an insect within a particular stage (Boivin and Belair 1989, Peters and Ehlers 1994). Pecan weevil larvae spend 1–2 yr in the soil; hence the development of resistance to soil-borne pathogens would be advantageous. Whether or not pecan weevil larvae develop resistance with age will have to be verified in future studies.

Several factors may limit the potential to control pecan weevil larvae with entomopathogenic nematodes. Foremost, entomopathogenic nematode virulence toward pecan weevil larvae does not appear to be great. Second, the cost of application is likely to be high relative to chemical insecticides. Multiple nematode applications may be necessary for pecan weevil larvae because they emerge over a 3- to 5-mo period (Harris 1985), and in certain systems, insect suppression from a single application of entomopathogenic nematodes may last only 2–4 wk (Duncan et al. 1996, Shapiro et al. 1996b).

Further research may overcome barriers to nematode control of pecan weevil larvae. Nematode application costs may be reduced through improvement of mass production, formulation and delivery technology (Grewal and Georgis 1998). Higher virulence may be found in nematode strains or species not yet tested. If no existing nematode strains show higher virulence then genetic improvement for virulence or other desired traits may be an option (Gaugler 1987). Genetic improvement of entomopathogenic nematodes has been demonstrated through artificial selection (Gaugler et al. 1989), hybridization (Shapiro et al. 1997), and genetic engineering (Hashmi et al. 1998). In addition to virulence, nematode persistence should also be sought to facilitate inoculative or introduction approaches to pecan weevil control. Even moderate levels of pecan weevil control may be cost-effective if the insect suppression is long-term. Although not common, persistent control using entomopathogenic nematodes has been reported in several systems (Klein and Georgis 1992, Parkman et al. 1994, Shields et al. 1999). Cropping systems with attributes such as stability, favorable soil conditions, and host availability throughout the year may be amenable to inoculative

approaches with entomopathogenic nematodes (Kaya 1990); the pecan weevil system fits these criteria.

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## References Cited

**Abbott, W. S. 1925.** A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

**Boivin, G., and G. Belair. 1989.** Infectivity of two strains of *Steinernema feltiae* (Rhabditida: Steinernematidae) in relation to temperature, age, and sex of carrot weevil (Coleoptera: Curculionidae) adults. J. Econ. Entomol. 82: 762–765.

**Bedding, R. A., and L. A. Miller. 1981.** Use of a nematode, *Heterorhabditis heliothidis,* to control black vine weevil, *Otiorhynchus sulcatus,* in potted plants. Ann. Appl. Biol. 99: 211–216.

**Bullock, R. C., R. R. Pelosi, and E. E. Killer. 1999.** Management of citrus root weevils (Coleoptera: Curculionidae) on Florida citrus with soil-applied entomopathogenic nematodes (Nematoda: Rhabditida). Fla. Entomol. 82: 1–7.

**Duncan L. W., and C. W. McCoy. 1996.** Vertical distribution in soil, persistence, and efficacy against citrus root weevil (Coleoptera: Curculionidae) of two species of entomogenous nematodes (Rhabditida: Steinernematidae: Heterorhabditidae). Environ. Entomol. 25: 174–178.

**Duncan, L. W., C. W. McCoy, and C. Terranova. 1996.** Estimating sample size and persistence of entomogenous nematodes in sandy soils and their efficacy against the larvae of *Diaprepes abbreviatus* in Florida. J. Nematol. 28: 56–67.

**Dutcher, J. D., and J. A. Payne. 1985.** The impact of pecan weevil control strategies on non-target arthropods, pp. 39–50. *In* W. W. Neel [ed.], Pecan weevil: research perspective. Quail Ridge Press, Brandon, MS.

**Ellis, H. C., P. Bertrand, and T. F. Crocker. 2000.** Georgia pecan pest management guide. Univ. Ga. Coop. Ext. Serv. Bull. 841.

**Gaugler, R. 1987.** Entomogenous nematodes and their prospects for genetic improvement. *In* K. Maramorosch [ed.], Biotechnology in Invertebrate Pathology and Cell Culture, pp. 457–484. Academic, San Diego, CA.

**Gaugler, R., J. F. Campbell., and T. R. McGuire. 1989.** Selection for host-finding in *Steinernema feltiae.* J. Invertebr. Pathol. 54: 363–372.

**Georgis, R., and R. Gaugler. 1991.** Predictability in biological control using entomopathogenic nematodes. J. Econ. Entomol. 84: 713–720.

**Glazer, I. 1992.** Invasion rate as a measure of infectivity of steinernematid and heterorhabditid nematodes to insects. J. Invertebr. Pathol. 59: 90–94.

**Grewal, P., and R. Georgis. 1998.** Entomopathogenic nematodes. *In* F. R. Hall and J. J. Menn [eds.], Methods in biotechnology, vol. 5. Biopesticides: use and delivery. Humana Press, Totowa, NJ.

**Harp, S. J., and H. W. Van Cleave. 1976.** New Records of natural enemies of the pecan weevil. Southwest. Entomol. 1: 38.

**Harris, M. K. 1985.** Pecan phenology and pecan weevil biology and management, pp. 51–58. *In* W. W. Neel [ed.], Pecan weevil: research perspective. Quail Ridge Press, Brandon, MS.

**Harris, M. K. 1999.** Pecan weevil management considerations, pp. 66–73. *In* B. McCraw, E. H. Dean, and B. W. Wood [eds.], Pecan industry: current situation and future challenges, third national pecan workshop proceedings. U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD.

**Hashmi, S., G. Hashmi, I. Glazer, and R. Gaugler. 1998.** Thermal response of *Heterorhabditis bacteriophora* transformed with the *Caenorhabditis elegans* hsp70 encoding gene. J. Exp. Zool. 281: 164–170.

**Hominick, W. M., B. R. Briscoe, F. G. del Pino, J. Heng, D. J. Hunt, E. Kozodoy, Z. Mracek, K. B. Nguyen, A. P. Reid, S. Spiridonov, and others. 1997.** Biosystematics of entomopathogenic nematodes: current status, protocols, and definitions. J. Helminthol. 71: 271–298.

**Kaya, H. K. 1990.** Soil ecology, pp. 93–116. *In* R. Gaugler and H. K. Kaya [eds.], Entomopathogenic nematodes in biological control. CRC, Boca Raton, FL.

**Kaya, H. K., and R. Gaugler. 1993.** Entomopathogenic nematodes. Annu. Rev. Entomol 38: 181–206.

**Klein, M. G. 1990.** Efficacy against soil-inhabiting insect pests, pp. 195–214. *In* R. Gaugler, and H. K. Kaya [eds.], Entomopathogenic nematodes in biological control. CRC, Boca Raton, FL.

**Klein, M. G., and R. Georgis. 1992.** Persistence of control of Japanese beetle (Coleoptera: Scarabaeidae) larvae with steinernematid and heterorhabditid nematodes. J. Econ. Entomol. 85: 727–730.

**Mannion, C. M., and R. K. Jansson. 1992.** Comparison of ten entomopathogenic nematodes for control of sweetpotato weevil (Coleoptera: Apionidae). J. Econ. Entomol. 85: 1642–1650.

**Mannion, C. M., H. E. Winkler, D. I. Shapiro, and T. Gibb. 2000.** Interaction between halofenozide and the entomopathogenic nematode *Heterorhabditis marelatus* for control of Japanese beetle (Coleoptera: Scarabaeidae) larvae. J. Econ. Entomol. 93: 48–53.

**Mizell, R. F. 1985.** Risk rating: A fruitful approach to management of the pecan weevil, pp. 69–78. *In* W. W. Neel [ed], Pecan weevil: research perspective. Quail Ridge Press, Brandon, MS.

**Morris, O. N., and V. Converse. 1991.** Effectiveness of steinernematid and heterorhabditid nematodes against noctuid, pyralid, and geometrid species in soil. Can. Entomol. 123: 55–61.

**Nyczepir, A. P., J. A. Payne, and J. J. Hamm. 1992.** Heterorhabditis bacteriophora: a new parasite of pecan weevil *Curculio caryae.* J. Invertebr. Pathol. 60: 104–106.

**Parkman, J. P., J. H. Frank, K. B. Nguyen, and G. C. Smart, Jr. 1994.** Inoculative release of *Steinernema scapterisci* (Rhabditida: Steinernematidae) to suppress pest mole crickets (Orthoptera: Gryllotapidae) on golf courses. Environ. Entomol. 23: 1331–1337. 16: 987–989.

**Patterson Stark, J. E., and L. A. Lacey. 1999.** Susceptibility of western cherry fruit fly (Diptera: Tephritidae) to five species of entomopathogenic nematodes in laboratory studies. J. Invertebr. Pathol. 74: 206–208.

**Peters, A. and R.-U. Ehlers. 1994.** Susceptibility of leatherjackets (*Tipula paludosa* and *Tipula oleracea;* Tipulidae; Nematocera) to the entomopathogenic nematode *Steinernema feltiae.* J. Invertebr. Pathol. 63: 163–171.

**Poinar, G. O. 1990.** Biology and taxonomy of Steinernematidae and Heterorhabditidae, pp. 23–62. *In* R. Gaugler and H. K. Kaya [eds.], Entomopathogenic nematodes in biological control. CRC, Boca Raton, FL.

**SAS Institute. 1985.** SAS user's guide: version 5 ed. SAS Institute, Cary, NC.

**Schirocki, A., and N.G.M. Hague. 1997.** Evaluation of UK heterorhabditids and steinernematids for the control of the black vine weevil (*Otiorhynchus sulcatus*). Ann. Appl. Biol. 18: 46–47.

**Shanks, C. H., and F. Agudelo-Silva. 1990.** Field pathogenicity and persistence of heterorhabditid and steinernematid nematodes (Nematoda) infecting black vine weevil larvae (Coleoptera: Curculionidae) in cranberry bogs. J. Econ. Entomol. 83: 107–110.

**Shapiro, D. I., I. Glazer, and D. Segal. 1996a.** Trait stability and fitness of the heat tolerant entomopathogenic nematode *Heterorhabditis bacteriophora* IS5 strain. Biol. Control 6: 238–244.

**Shapiro, D. I., G. L. Tylka, and L. C. Lewis. 1996b.** Effects of fertilizers on virulence of *Steinernema carpocapsae.* Appl. Soil Ecol. 3: 27–34.

**Shapiro, D. I., I. Glazer, and D. Segal. 1997.** Genetic improvement of heat tolerance in *Heterorhabditis bacteriophora* through hybridization. Biol. Control 8: 153–159.

**Shapiro, D. I., J. R. Cate, J. Pena, A. Hunsberger, and C. W. McCoy. 1999.** Effects of temperature and host age on suppression of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) by entomopathogenic nematodes. J. Econ. Entomol. 92: 1086–1092.

**Shapiro, D. I., and C. W. McCoy. 2000.** Virulence of Entomopathogenic nematodes to *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in the laboratory. J. Econ. Entomol. 93: 1090–1095.

**Shields, E. J., A. Testa, J. M. Miller, and K. L. Flanders. 1999.** Field efficacy and persistence of the entomopathogenic nematodes *Heterorhabditis bacteriophora* 'Oswego' and *H. bacteriophora* 'NC' on alfalfa snout beetle larvae (Coleoptera: Curculionidae). Environ. Entomol. 28: 128–136.

**Simons, W. R. 1981.** Biological control of *Otiorhynchus sulcatus* with heterorhabditid nematodes in the glasshouse. Neth. J. Plant Pathol. 87: 149–158.

**Smith, M. T., R. Georgis, A. P. Nyczepir, and R. W. Miller. 1993.** Biological control of the pecan weevil, *Curculio caryae* (Coleoptera: Curculionidae), with entomopathogenic nematodes. J. Nematol. 25: 78–82.

**Stuart, R. J., and R. Gaugler. 1996.** Genetic adaptation and founder effect in laboratory populations of the entomopathogenic nematode *Steinernema glaseri.* Can. J. Zool. 74: 164–170.

**Tanada, K., and J. R. Fuxa. 1987.** The pathogen population, pp. 113–157. *In* Fuxa and Tanada [eds.], Epizootiology of insect diseases. Wiley, New York.

**Tanada, Y., and H. K. Kaya. 1993.** Insect pathology. Academic, San Diego, CA.

**Tedders, W. L. 1973.** Pecan weevil: suppression of larvae with the fungi *Metarhizium anisopliae* and *Beauveria bassiana* and the nematode *Neoaplectana dutkyi.* J. Econ. Entomol. 66: 723–725.

**Woodring, J. L., and H. K. Kaya. 1988.** Steinernematid and heterorhabditid nematodes: A handbook of biology and techniques. Southern Cooperative Series Bulletin 331. Arkansas Agricultural Experiment Station, Fayetteville, AR.

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